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Canaigre Investigations†

III. An Improved Method of Extraction*

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Introduction

During World War II, acute shortages of vegetable tanning materials from both domestic and foreign sources developed. It was expected that when the war ended, these shortages would be less severe. It now appears, however, that this is not the case. Imports of some of these materials are still short, and our supplies of chestnut wood are continually becoming more limited and inaccessible, and of lower quality. This country is thus faced with the problem of greater dependence upon foreign tannins, the increased use of the more expensive synthetic tannins, or the development of more adequate supplies of the low-cost natural vegetable tannins. The Hides, Tanning Materials and Leather Division of the Eastern Regional Research Laboratory is actively engaged in a program[6] on the economic production and processing of new and undeveloped domestic vegetable tannins. Any marked accomplishments in this field will materially aid in meeting the tannin needs of the leather industry. The report of further progress in the development of canaigre, one of the native tanning materials now under study, should therefore be particularly welcome to tanners at this time.

Although canaigre received much attention as a potential source of tannin during a period of about 30 years—from 1880 to 1910—it never became established as a commercial tanning agent. Rogers and Russell[8] have reviewed the early work on canaigre and suggested possible reasons why its use was not generally adopted commercially.

Utilization of canaigre for production of commercially valuable tanning extracts presents problems of growing, harvesting, processing, salvaging by-products, and marketing. Problems involved in growing and harvesting are being studied as a cooperative project with the Bureau of Plant Industry, Soils and Agricultural Engineering. Processing studies are being conducted at the Eastern Regional Research Laboratory.

In processing by normal leaching methods the high starch content and

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high water holding capacity of canaigre roots render efficient extraction difficult. This paper reports the results of studies of extraction procedures and the development of a more efficient method.

Extraction Studies

With canaigre as with other tannin-containing materials, the state of subdivision is important in determining the time required and the effectiveness of water extraction. Freshly dug canaigre roots can be readily sliced, diced or shredded, and when dry they can be powdered satisfactorily. Thinly sliced roots in small quantities can be extracted fairly well with water, provided the temperature is maintained below 65° C. Prepared in this manner they have been used for analytical extractions in a Reed-Churchill[5] extractor. Shredded roots can also be extracted under the same conditions, but recovery of tannin is not so efficient as in the extraction of thin slices. At temperatures above the gelatinization point of starch, which is about 70° C., it is impossible to make a satisfactory water extraction of either sliced or shredded roots, even in analytical size lots. Powdered material cannot be extracted even when small samples are used at room temperature.

Countercurrent Leaching in Open Leaches.—An experiment is cited to illustrate the results obtained in extraction of shredded canaigre roots by a battery of open glass leaches operated countercurrently. The operation simulated that of industrial equipment in most respects, and in previous tests with other materials had given results comparable with those obtained in industry. The leach vats, however, were small and shallow, being only about 8 inches in diameter and 1 foot deep.

The battery consisted of 10 vats immersed in a water bath maintained at approximately 50° C. The canaigre used came from a bulk lot of wild Arizona roots which had been shredded in a Kummer Shredder[7] and air dried. Each vat contained 1200 grams of air-dried shredded root material and 6 liters of water or liquor. Once each hour the liquors were removed from each vat by siphoning and moved forward to the next vat nearer the head. Once every two hours a head liquor was removed from the system, a spent leach removed, and a new head leach installed. During the intervals when liquors were not being moved forward they were circulated in each vat by air lifts. This leaching system was operated until a total of 13 head liquors had been taken off. It was then closed down by not adding new head leaches when spent leaches were removed. Since the analyses of the last 7 head leach liquors showed them to be uniform in composition, it was concluded that equilibrium had been established in the leaching system and the analyses of these liquors could be safely used for calculation of leaching efficiency. The data given in Table I show the rather low leaching efficiencies

TABLE I

COUNTERCURRENT LEACHING OF SHREDDED ARIZONA CANAIGRE IN LABORATORY OPEN LEACHES*

	Amount	Total Solids†		Soluble Solids†	Insolubles	Non Tannin	Tannin		Purity (Tan/SS)
		Per cent	Per cent based on original material	Per cent	Per cent	Per cent	Per cent	Per cent based on original material	
Original material‡	7,612 g.	52.0	......	45.3	6.7	18.9	26.4	......	58.3
Recovered liquor (average)	21,780 ml.	11.7	64(EL)§	11.4	0.3	5.8	5.6	61(EL)§	49.1
Spent material‡	4,838 g.	12.3	......	11.6	0.7	2.3	9.3	......	80.2
Total in recovered liquor and spent material	....	....	79	....	...	....	....	83	....

*Extractions for tannin analysis were made in an extractor having an outside cooling chamber that permitted refluxing and maintaining extraction temperature at approximately 60° C. Tannin analyses were made by the Official A.L.C.A. method[1].

†Total solids and soluble solids when referred to original or spent materials signify total extractives and soluble extractives, respectively.

‡All results were calculated on a moisture-free basis.

§EL = efficiency of leaching.

TABLE II

EFFECT OF STEAMING AND AMYLASE TREATMENT ON CANAIGRE*

	Total Solids	Soluble Solids	Insolubles	Non Tannin	Tannin	Purity (Tan/SS)	Reducing Sugars	Total Sugars
	Per cent	Per cent	Per cent	Per cent	Per cent		Per cent	Per cent
New Mexico sliced roots								
Original material (control)	59.1	57.5	1.6	35.1	22.4	39.0	6.3	23.7
After steaming†	63.1	58.0	5.1	36.4	21.6	37.2	5.5	24.4
After steaming and amylase treatment‡	67.1	65.3	1.8	42.1	23.2	35.5	7.9	29.0
Arizona shredded roots								
Original material (control)	48.8	47.5	1.3	22.4	25.1	52.8	...	13.1
After steaming and amylase treatment§	76.8	71.2	5.6	43.4	27.8	39.1	...	27.0

*Tannin was extracted in Reed-Churchill extractor at 65° C. Tannin analyses were made by the Official A.L.C.A. method[1].

†Ten times the sample weight of water was added, and the material was steamed for 1 hour in an Arnold sterilizer.

‡Ten times the sample weight of water was added, and the material was steamed for 1 hour; it was then cooled to 55° C., and 0.5 per cent amylase based on total weight of sample was added, and the mixture was held for 5 hours.

§Ten times the sample weight of water was added, and the material was steamed for 2 hours; it was then cooled to 55° C., and 1.0 per cent amylase was added. This mixture was held for 2.5 hours.

btained. The leaching efficiency was 64 per cent on a total solids basis and 61 per cent on a tannin basis.

It is probable that these leaching results are more favorable than would be obtained in practice, as leaching large quantities of canaigre involves difficulties not present in leaching on a laboratory scale. One is the swelling and packing of sliced or shredded material in the leaches, which makes percolation of water difficult or nearly impossible. A further test was conducted to determine the effect of leaching in vats of a height comparable with that used in industry. A wooden tank about 1 foot square, 8 feet tall, and fitted with a false bottom was filled with shredded canaigre. Cold water run in at the top percolated readily, but when water at 60° to 65 ° C. was run in, the flow soon became so restricted that satisfactory leaching was impossible. From these results it seems evident that sliced or shredded canaigre could not be effectively leached with warm water in the tall leaches customarily used commercially.

Since satisfactory extraction of tannin from canaigre by the usual leaching procedures is not possible in the presence of starch, it is necessary to remove the starch before extraction of the tannin or to develop extraction methods in which starch would not interfere. Starch may be removed mechanically or by hydrolysis with acids or enzymes. Mechanical removal would be impractical because of the large amount of water required, and acid hydrolysis would destroy tannin. Enzymatic hydrolysis, however, seemed to offer possibilities.

Enzymatic Hydrolysis before Tannin Extraction.—Preliminary studies howed that after treatment with a fungus amylase preparation canaigre was much more readily extracted. After such treatment samples of finely powdered material were successfully extracted with Reed-Churchill equipment.

Although certain information concerning the action of amylase on canaigre starch was lacking, it was thought advisable to determine whether, after such treatment, canaigre would be suitable for leaching. Accordingly the following test was made. Ten liters of water were added to 3 kilograms of shredded Arizona canaigre. After steaming for 3 hours and cooling to 55° C., 1 per cent of fungus amylase was added. The temperature was maintained at 55° C. for one hour, then the mixture was placed in a constant temperature room at 30° C., where it was kept overnight. It was inoculated with a bacterium HA,[4] and the liquor was pumped over the shredded roots by means of air lifts. After 48 hours' fermentation the temperature was raised to 55° C. and the liquor removed. Water was pumped over the material for one hour and siphoned off. This leaching operation was repeated until a total of nine liquors had been taken off over a period of three days, the intervals

TABLE III

CENTRIFUGAL EXTRACTION OF CANAIGRE*

Experiment No.	Treatment	Material	Amount	Total Solids†		Soluble Solids†	Insolubles	Non Tannin	Tannin		Purity (Tan/SS)
				Per cent	Per cent based on original material	Per cent	Per cent	Per cent	Per cent	Per cent based on original material	
			Arizona Roots, Shredded								
1	No pretreatment; stirred for 30 min. at 40-45° C.; 5 washes.	Original material‡......	1,356 g.	52.0	....	45.3	6.7	18.9	26.4	....	58.3
		Recovered liquor.......	12,960 ml.	3.6	66(EE)§	3.4	0.2	1.6	1.8	65(EE)§	52.9
		Spent material‡........	840 g.	14.1	....	11.4	2.7	1.6	9.8	....	86.0
		Total in recovered liquor and spent material.....	.....	....	83	....	....	....	....	88	....
2	Crushed between rolls at 0.005 inch; stirred for 30 min. at 40-46° C.; 5 washes.	Original material‡......	2,750 g.	52.0	....	45.3	6.7	18.9	26.4	....	58.3
		Recovered liquor.......	27,180 ml.	4.3	82(EE)§	3.9	0.4	1.8	2.1	79(EE)§	53.8
		Spent material‡........	1,402 g.	9.4	....	9.0	0.4	2.9	6.1	....	68.2
		Total in recovered liquor and spent material.....	.....	....	91	....	....	....	....	90	....
3	Crushed between rolls at 0.005 inch; stirred for 30 min. at 45-50° C.; 6 washes.	Original material‡......	2,747 g.	52.0	....	45.3	6.7	18.9	26.4	....	58.3
		Recovered liquor.......	30,990 ml.	4.6	100(EE)§	4.1	0.5	2.1	2.0	85(EE)§	48.8
		Spent material‡........	1,208 g.	9.3	....	8.5	0.8	3.6	4.9	....	57.6
		Total in recovered liquor and spent material.....	.....	....	108	....	....	....	....	94	....
4	Ground to pass 1-mm. screen; stirred for 30 min. at 40-45° C.; 5 washes.	Original material‡......	1,346 g.	52.0	....	45.3	6.7	18.9	26.4	....	58.3
		Recovered liquor.......	13,680 ml.	3.9	76(EE)§	3.7	0.2	1.6	2.1	81(EE)§	56.8
		Spent material‡........	728 g.	11.5	....	10.4	1.1	2.2	8.2	....	78.8
		Total in recovered liquor and spent material.....	.....	....	88	....	....	....	....	98	....
5	Pulped for 5 min.; stirred for 25 min. at 40-45° C.; 5 washes.	Original material‡......	1,359 g.	52.0	....	45.3	6.7	18.9	26.4	....	58.3
		Recovered liquor.......	16,830 ml.	3.4	81(EE)§	3.2	0.2	1.4	1.8	84(EE)§	56.3
		Spent material‡........	738 g.	13.4	....	9.3	4.1	1.9	7.4	....	79.6
		Total in recovered liquor and spent material.....	.....	....	95	....	....	....	....	100	....

		New Mexico Roots, Sliced or Shredded										
6	Sliced roots; no pretreatment, stirred for 30 min. at 40-45° C.; 5 washes.	Original material‡......	1,366 g.	51.6	....	50.9	0.7	23.6	27.3	....	53.6
		Recovered liquor.......	13,190 ml.	4.7	88(EE)§	4.6	0.1	2.4	2.2	78(EE)§	47.8
		Spent material‡........	687 g.	10.0	....	9.4	0.6	2.6	6.8	....	72.3
		Total in recovered liquor and spent material.....	.....	....	98	....	....	....	....	90	....
7	Sliced roots; crushed; stirred for 30 min. at 40-45° C.; 5 washes.	Original material‡......	1,304 g.	54.0	....	50.8	3.2	24.4	26.4	....	52.0
		Recovered liquor.......	13,450 ml.	4.6	88(EE)§	4.4	0.2	2.3	2.1	82(EE)§	47.7
		Spent material‡........	655 g.	8.7	....	8.3	0.4	2.4	5.9	....	71.1
		Total in recovered liquor and spent material.....	.....	....	96	....	....	....	....	93	....
8	Shredded roots; pulped for 5 min.; stirred for 25 min. at 43-45° C.; 5 washes.	Original material‡......	1,412 g.	48.7	....	45.6	3.1	22.6	23.0	....	50.4
		Recovered liquor.......	16,960 ml.	4.0	99(EE)§	3.6	0.4	2.1	1.5	78(EE)§	41.7
		Spent material‡........	678 g.	10.7	....	9.9	0.8	2.4	7.5	....	75.8
		Total in recovered liquor and spent material.....	.....	....	109	....	....	....	....	94	....

*Extractions for tannin analysis were made in an extractor having an outside cooling chamber that permitted refluxing and maintaining extraction temperature at approximately 60° C. Tannin analyses were made by the Official A.L.C.A. method (1).

†Total solids and soluble solids when referred to original or spent materials signify total extractives and soluble extractives, respectively.

‡All results were calculated on a moisture-free basis.

§EE = efficiency of extraction.

between removal of liquors ranging from 1 to 4 hours. The temperature was gradually increased until for the ninth liquor it was 80° C.

The calculated leaching efficiency was 87.5 per cent on the tannin basis. On analysis, the powdered extract prepared from the composite liquor showed 51.9 per cent tannin, 2.5 per cent sugar and 14 per cent insolubles and had a purity of 65.6 on a soluble solids basis. These results indicate that after amylase treatment this material could be leached satisfactorily on a small scale. The extract produced was satisfactory except for its high insolubles content.

These results seemed encouraging, but further investigation of the action of amylases on canaigre starch showed that in order to effect fairly complete hydrolysis of the starch, 7.5 per cent of a highly purified fungus amylase, on the basis of the total canaigre, was required. Cheap sources of amylase such as malted barley or mold bran are not suitable because the large quantities required would increase the non tannin markedly, and decrease the tannin by precipitation with the proteinaceous materials present. The excessively large quantities of purified amylase required are no doubt due to its inactivation by tannin.[2]

Further objections to the use of amylase are that it produces increased quantities of insolubles and unfermentable non tannins. These disadvantages are partially offset by the increased tannin yields after amylase treatment. The effects of preheating and amylase treatment are illustrated in Table II.

Treatment of sliced New Mexico canaigre with 0.5 per cent of its weight of purified fungus amylase results in an increase of 5.3 per cent in total sugar, 1.6 per cent in reducing sugar and 7.0 per cent in non tannins. The tannin increased slightly, but the purity decreased from 39.0 to 35.5. One per cent amylase added to Arizona canaigre caused a much more marked effect. Sugars and non tannins were almost doubled, and the purity decreased from 52.8 to 39.1. Actually hydrolysis was not complete in either case. The New Mexico sample contained more than 21 per cent starch, which on hydrolysis would give about 23 per cent sugar as glucose. This plus the 23.7 per cent present in the original material would give about 47 per cent total sugar, whereas only 29.0 per cent was present. Similarly, the Arizona sample contained 19 per cent starch, which, if completely hydrolyzed to glucose, would give a total sugar value of about 34 per cent. Only 27 per cent was found.

Centrifugal Countercurrent Extraction.—Since neither the usual leaching technique nor the amylase pretreatment gave satisfactory results, a new method of extraction was devised in which the presence of starch does not interfere. By its use more than 80 per cent of the tannin is recovered in the leach liquors. In this procedure the canaigre is suitably prepared for extraction by crushing between stainless steel rolls, grinding to a fine powder, or wet pulping. Extraction is carried out countercurrently by a succession of treatments with water (or liquor) alternated with removal of liquor by means of a basket-type centrifuge. Since this extraction method has been described in detail in a previous publication,[3] only a few modifications in the disintegration technique will be discussed here.

Numerous experiments have been carried out with this technique to determine conditions necessary for the production of leach liquors of satisfactory quality and for high leaching efficiency. These studies have included variations in the preparation of material and the temperature and time of mixing. The results of several representative tests are recorded in Table III.

Preliminary tests demonstrated that when a heavy filter cloth of the type used in filter presses was employed in the centrifuge basket, 50° C. was the highest temperature that could be safely used without clogging by swollen starch grains. Even this temperature was too near the critical point, and most of the subsequent tests were run at 40° to 45° C.

The length of time that water or liquor was mixed with the root material was of little importance provided that the roots were in a finely divided state. Fifteen minutes' mixing gave as good tannin recovery as 2 hours. Probably even less than 15 minutes would suffice, but it was the shortest time that could be used with the laboratory equipment and allow for the necessary manipulation. The 30 minute period used in these tests was selected because it allowed sufficient time for measuring liquors, centrifuging, etc.

In Experiment 1, Table III, the coarsely shredded roots were extracted without any pretreatment. Tannin recovery was considerably better than that obtained by ordinary leaching (see Table I), but only 65 per cent of that originally present was recovered. Much better results were obtained by moistening the shredded roots and crushing between stainless steel rolls set 0.005 of an inch apart (Experiment 2, Table III). Tannin recovery was increased to 79 per cent of that present in the original material. A still higher extraction efficiency of 85 per cent was obtained with the crushed material if the temperature while mixing was held near the workable maximum of 50° C. and one more wash water was added to the system. The quality of the recovered liquor, however, was much lower, and the purity dropped from 53.8 to 48.8. The extracts made from the liquor were cloudy and of poor quality. It seems doubtful whether the additional tannin recovered would compensate for the decrease in quality.

The extraction efficiency obtained when powdered roots were extracted by this procedure was 81 per cent on a tannin basis (Experiment 4, Table III). This represents good tannin recovery, but the reduction of roots to a fine powder is a rather expensive operation. Further study demonstrated that even better results could be obtained by employing a wet pulping process. The machine used has a violent stirring and cutting action. The shredded roots were reduced to a pulp by 5 minutes' treatment and were then stirred with an electric stirrer for 25 minutes before extraction in the centrifuge. The pulping operation was carried out only when the shredded roots were first added to the system. Tannin recovery by this method was 84 per cent of that originally present (Experiment 5, Table III). No doubt other types of pulping equipment could be used with equal efficiency.

Since sliced roots are usually used for analytical purposes, it was thought desirable to determine what recovery could be obtained when material in this form, without pretreatment, was used with the centrifuging technique. The results of these tests are recorded in Table III, Experiments 6 and 7. The tannin recovered when sliced roots were used amounted to 78 per cent of that originally present. The tannin recovery was increased to 82 per cent when the sliced roots were crushed between steel rolls with a clearance of 0.005 of an inch before extraction. Sliced roots appear to have no advantage over shredded roots and are much more expensive.

In Experiment 8, Table III, results of extraction studies on New Mexico shredded roots are recorded. The tannin recovery, after wet pulping and centrifugal extraction, was 78 per cent, as compared with 84 per cent obtained with Arizona roots. These results illustrate unexplained differences in composition and reactions of roots from New Mexico and Arizona.

Table III shows that the spent materials still contain relatively large quantities of tannin. However, since approximately half the original material has been removed by extraction, on the basis of the original material, these quantities would be about halved. Furthermore, from the results of Experiment 3 it would appear that the removal of the last portions of tannin may not be advantageous, since other undesirable solubles which lower the quality of the product are also obtained.

Table III shows that in some instances there are more extractives in recovered liquors and spent materials than are shown to be present by the analysis of the original material. This discrepancy may be explained by the present unsatisfactory method of analysis of the spent material, by incomplete analytical extraction of the original material or by a combination of these two factors. In previous extraction tests of other materials, this same anomaly has apparently been due mainly to the first mentioned cause. In this case, however, supplementary tests have shown that this effect may have occurred as the result of incomplete extraction of the original canaigre sample for analysis.* Abnormally low values are due to incomplete extraction of spent materials. Studies for the improvement of methods of analysis for canaigre are now under way.

* Analytical extractions for the determination of tannin, except where otherwise noted, were made by procedure given in a footnote in Table I.

While the calculation of extraction efficiencies in the manner indicated is of value in comparing different extraction techniques, a more pertinent figure from the standpoint of the extract manufacturer is the amount of available tannin. It is seen that with the wet pulping procedure the lot of canaigre from Arizona reported in Table III, Experiment 5, yielded 22.9 per cent of available tannin, certainly a high figure, as compared with several materials now in use.

From the laboratory data thus far obtained, it appears that some type of wet pulping followed by extraction with a centrifuge, or other equipment that gives efficient separation of liquors from the solids, would produce satisfactory results on a commercial scale. Pilot plant studies are now being started.

Discussion

Canaigre roots differ widely from the barks and woods now serving as the principal sources of vegetable tannin. Because of this, it has been necessary, in order to obtain acceptably efficient tannin recovery, to employ unusual and more forceful methods of extraction, which are radical departures from the leaching methods now in general use. The laboratory equipment available for these tests was cumbersome and not adapted to large-scale production. However, plant-scale equipment, such as pulping machines, continuous centrifuges and continuous vacuum filters, which would make the process continuous or nearly so, is available. Work is now under way in the pilot plant to determine the feasibility of the process and to obtain data on the cost of operation.

A systematic study of the starch content of canaigre roots has not yet been made, but the few samples examined have shown that starch in the moisture-free mature roots may range from 20 to 44 per cent. Since extraction and leaching studies, in which very little starch is removed, yield about 50 per cent of water solubles, it is probable that the starch content of the spent canaigre roots would be nearly double that of the dry roots before extraction. It is possible that this starch might be separated and used as such, or, together with other carbohydrates in the spent material, hydrolyzed and fermented to yield valuable by-products. This phase of the problem will receive further study.

The advantages of a domestic tannin crop that can be easily and quickly produced are obvious. It would materially aid in reducing our country's dependence upon foreign supplies and would justify the whole-hearted cooperation and support of the tanning industry.

Summary

A method for extracting tannin from canaigre has been devised which gives yields much higher than those obtained with the current leaching pro-

cedures normally used for other materials. It consists of wet pulping and mixing under controlled temperature conditions and removal of the liquor from the solids by centrifugal separation. As far as can be judged from these tests, this process could be readily adapted to plant-scale operation.

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